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Healthy Puppies and Kittens as Carriers of *Campylobacter* spp., with Special Reference to *Campylobacter upsaliensis*

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Living in a household with a dog or cat has previously been identified as a significant risk factor for acquiring campylobacteriosis, in particular, with reference to *Campylobacter upsaliensis* infection. In a cross-sectional study carried out in Denmark between August and December 1996, 72 healthy puppies and 42 healthy kittens, aged between 11 and 17 weeks, were sampled for fecal campylobacter shedding by culture of rectal swab specimens on blood-free agar base with cefoperazone at 32 mg/liter and amphotericin at 10 mg/liter and on blood-free agar base with cefoperazone at 8 mg/liter, teicoplanin at 4 mg/liter, and amphotericin at 10 mg/liter. Additionally, with respect to the *C. upsaliensis* transmission potential of poultry, a chicken cloacal swab sample from each of 100 different broiler flocks was included in the study for comparison. We found 21 (29%) of the puppies positive for *Campylobacter* spp., with a species distribution of 76% *C. jejuni*, 5% *C. coli*, and 19% *C. upsaliensis*. Of the kittens examined, two (5%) excreted campylobacters; both strains were *C. upsaliensis*. None of the chicken samples examined were found to be positive for *C. upsaliensis*. We concluded that young puppies and kittens are potential transmitters of human-pathogenic *Campylobacter* spp., including *C. upsaliensis*, while poultry seems negligible in *C. upsaliensis* epidemiology.

The majority of cases of human campylobacteriosis in developed countries are most probably caused by consumption of undercooked poultry, raw milk, or untreated surface water, while the remaining incidences may be assigned to a multitude of other sources (1, 23). According to some risk analyses, living in a household with a dog or cat is a considerable risk factor (5, 11, 14, 22), and investigations of healthy pets have revealed carrier rates of 10 to 29.6% in dogs (4, 9, 13, 16) and 14.5 to 32% in cats (4, 9). According to others (21), only diarrheic animals pose a risk. Certainly, a positive correlation has been found between diarrhea and campylobacter excretion in young dogs (4, 7–9), while the excretion rates in adults seem to be approximately equal whether they are diarrheic or healthy (4, 9, 12, 16).

The *Campylobacter* species most frequently isolated from human cases of campylobacteriosis has been *Campylobacter jejuni*, but several reports in the late 1980s and early 1990s have stated the significance of *C. upsaliensis* in human gastroenteritis and cases of septicemia (2, 10, 15, 20). Apart from humans, *C. upsaliensis* has been reported to occur only in dogs and cats (17), with prevalence rates of 28 to 82% (4, 16), which has focused attention on dogs and cats in the epidemiology of *C. upsaliensis* infection.

Puppies and kittens, which often live in close proximity to humans, with the probability of direct transmission of pathogens, have not been thoroughly examined with respect to *Campylobacter* sp. carriage. This paper reports on a cross-sectional study on the *Campylobacter* sp. carriage rates of healthy puppies and kittens approximately 3 month old.

Totals of 72 puppies and 42 kittens, aged between 11 and 17 weeks, were randomly selected when admitted to 12 participating small-animal veterinary clinics for routine health examination and vaccination between August and December 1996. A maximum of one pet per household was included in the study. A rectal swab specimen was obtained from each animal and forwarded on the same day to our laboratory in an airtight test tube with Cary-Blair transport medium (Oxoid). In addition, a chicken cloacal swab from each of 100 different broiler flocks was included in the study for comparison of the strain distribution patterns of pets and poultry, with special reference to *C. upsaliensis*.

The swabs were streaked directly onto modified CCDA (mCCDA) plates (blood-free agar base with cefoperazone at 32 mg/liter and amphotericin at 10 mg/liter) (Oxoid CM 739/SR 155) and CAT plates (blood-free agar base with cefoperazone at 8 mg/liter, teicoplanin at 4 mg/liter, and amphotericin at 10 mg/liter) (Oxoid CM 739/SR 174). The inoculated plates were incubated in a microaerophilic atmosphere for 48 h, mCCDA plates at 42°C and CAT plates at 37°C. The microaerophilic atmosphere was created by flushing of the incubation jars with an N_2-H_2-CO_2 gas mixture (65%:25%:10%) for 1 min immediately before closure. Campylobacter-like colonies from the selective plates were subcultured one or more times on blood agar until pure. Bacterial colonies exhibiting curved or spiral motile rods as viewed under phase-contrast microscopy were presumptively identified as *Campylobacter* spp. Further species identification comprised tests for catalase production, hippurate and indoxylacetate hydrolysis, and susceptibility to cephalothin and nalidixic acid, in accordance with standard procedures (18, 19). The type strains *C. jejuni* CCUG 11284, *C. coli* CCUG 11283, and *C. upsaliensis* CCUG 14913 were included as positive controls.

Of 72 puppies, 21 (29%) were found positive for *Campylobacter* spp. with a species distribution of 76% (16 isolates) *C. jejuni*, 5% (1 isolate) *C. coli*, 19% (4 isolates) *C. upsaliensis*, and no *C. lari*. Of 42 kittens, 2 (5%) were positive for a *Campylobacter* sp.; both isolates were *C. upsaliensis*. Of 100 poultry samples, 64 (64%) were positive for *Campylobacter* spp., with a species distribution of 87% (56 isolates) *C. jejuni* and 13% (8 isolates) *C. coli.*

The isolation frequency ratios (IFR), i.e., the number of positive isolations on the mCCDA plate series at 42°C versus

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that of the CAT plate series at 37°C, were as follows: $IFR_{C.\ jejuni}$, 1.4 (62/45); $IFR_{C.\ coli}$, 3.0 (9/3); $IFR_{C.\ upsaliensis}$, 1.7 (5/3).

We are not aware of any published studies on campylobacter carriage in randomly selected healthy young puppies, but a few studies of pet dogs below 1 year of age have been carried out, reporting 20 to 23.8% carriage rates (9, 13), slightly lower than but comparable to our present result of 29%. Most risk analyses have dealt with pets of mixed ages, but Salfield and Pugh found a significant risk for children aged 0 to 5 years of acquiring campylobacteriosis by living with a puppy in the family (22). Even though cats have been identified as a risk factor too, we found that the healthy kittens examined in this study were rather low-prevalence campylobacter carriers (5%). This finding is in accordance with that of Gondrosen et al., who found 0 of 12 healthy kittens examined infected (9).

Chicken differed from dogs and cats in species distribution. They had, as expected, a high prevalence of *C. jejuni* and were negative for *C. upsaliensis*. Although poultry probably is responsible for a considerable number of human cases of campylobacteriosis, the present results demonstrate that poultry is not likely to contribute to *C. upsaliensis* epidemiology at all. The present low prevalence rate in healthy kittens (5%) makes a conclusion about strain distribution impossible, but the fact that only *C. upsaliensis* was isolated may inspire further studies of cats as a source of *C. upsaliensis*.

We recovered five *C. upsaliensis* isolates on mCCDA, while only three isolates were recovered on the CAT medium, although this medium is claimed to be virtually equivalent to filter methods that previously have been the methods of choice for isolation of *C. upsaliensis* (2, 3). However, in our study, it proved inferior to even mCCDA for *C. upsaliensis* isolation, but also for isolation of both *C. jejuni* and *C. coli*. Thus, the true rates of *C. upsaliensis* carriage by puppies and kittens might have been higher than our actual findings, because most authors consider media like mCCDA, with 32 mg of cefoperazone per liter, inferior to filter methods with respect to recovery of *C. upsaliensis* (2–4). In accordance with our results, however, a screening of human diarrheal cases for *Campylobacter* spp. by the Statens Serum Institut has revealed a low rate of *C. upsaliensis* isolation as well, even though a filter method was included (6), indicating, that *C. upsaliensis* might be of minor significance in campylobacter epidemiology in Denmark.

Complete understanding of the epidemiology of campylobacteriosis remains a challenge to researchers, although a considerable amount of knowledge has been acquired during the last 2 decades. In particular, the specific pet-to-human link requires further studies on the virulence of campylobacters isolated from nonclinical carriers and on the duration of the excretion period. From the results of this study, we conclude, however, that Danish puppies may be a considerable reservoir of the human pathogens *C. jejuni* and *C. upsaliensis*, while healthy kittens seem to be of limited significance. Further, we consider poultry without any significance in *C. upsaliensis* epidemiology.

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